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TWO TYPES OF NEURONES IN THE MYENTERIC PLEXUS OF DUODENUM IN THE GUINEA-PIG

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SUMMARY

1. Intracellular recordings have been made from neurones lying in the myenteric plexus of guinea-pig duodenum; some aspects of their membrane properties have been studied by passing current through the intracellular electrode while recording changes in membrane potential.

2. The current–voltage relationship was linear for small changes in membrane potential, input resistances ranging from 125 to 250 MΩ. Larger hyperpolarizing currents (causing changes of 20–40 mV) caused the input resistance to fall.

3. Depolarizing currents of 1 to 10×10^{-10} A initiated action potentials with amplitudes of up to 95 mV.

4. Two types of cell were distinguished when an action potential was initiated. In one group the action potential and undershoot had a form similar to that recorded from other mammalian ganglia. In the second group an action potential was followed by both an undershoot and a prolonged afterhyperpolarization which was associated with a decrease in cell resistance.

5. The two groups of cells were further distinguished by their responses to transmural stimulation. Only those cells which did not show an afterhyperpolarization could be shown to receive a synaptic input.

6. The mechanism by which each cell type generates an action potential was different. The action potentials recorded from cells which had a detectable synaptic input were abolished by tetrodotoxin. In contrast, those recorded from the other type of cells persisted in the presence of tetrodotoxin. Preliminary experiments suggest that during an action potential these cells become permeable to both sodium and calcium ions.

7. Abolition of the calcium component of the action potential in cells which generated an afterhyperpolarization abolished this latter potential.

8. The role of these two groups of cells is discussed.

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INTRODUCTION

There is evidence for the existence of several functionally different neurones within the wall of the gastrointestinal tract. These include sensory neurones, neurones which excite or inhibit its smooth muscles, and interneurones (Langley, 1922). Studies on the peristaltic and other reflexes of the tract suggest that these neurones are connected in a way which makes them capable of co-ordinating an orderly sequence of movements of the longitudinal and circular muscle layers (Kosterlitz, 1968). Thus the peristaltic reflex is unaffected when the extrinsic nerves to a segment of intestine are cut and allowed to degenerate (Bülbring, Lin & Schofield, 1958). This reflex is blocked by tetrodotoxin, local anaesthetics and ganglion blocking drugs and there is little doubt about its nervous origin.

The aim of the work reported here was to examine the properties and synaptic connexions of the neurones of the myenteric plexuses of guinea-pigs, using intracellular recording and stimulating techniques. Until recently (Nishi & North, 1973) the only information about the electrical properties of enteric neurones had been obtained from records made with extracellular electrodes (Wood, 1970; Ohkawa & Prosser, 1972*a*). The latter experiments were done on segments of the small intestine of cats. Of necessity, all the recorded units were spontaneously active; they could be driven only on rare occasions by electrical stimulation. Several types of neurones were distinguished according to the pattern of their spontaneous activity.

The experiments reported here were carried out on the myenteric plexus of the guinea-pig duodenum which was isolated from the circular layer but remained attached to the longitudinal layer of smooth muscle (Ambache, 1954). No evidence for pace-maker activity has been observed in any of the neurones recorded from so far (some 200). Two types of neurone have been distinguished in this preparation; those receiving extensive excitatory synaptic (probably cholinergic) input and those which could not be excited synaptically by gross electrical stimulation of the preparation. The latter neurones were characterized by a marked depression in excitability following the initiation of an action potential. Their function remains to be determined.

Preliminary accounts of some of this work have been published (Hirst, Holman, Prosser & Spence, 1972; Hirst & Spence, 1973).

METHODS

Experiments were carried out on the myenteric plexus which adhered to the longitudinal muscle layer after this had been stripped from segments of the duodenum of guinea-pigs, 350–550 g in weight (Ambache, 1954). Preparations were

pinned out on coverslips which had been coated with a thin layer of silicone rubber (Sylgard, 194; Dow Corning Ltd), using pins cut from 40 μm tungsten wire. The coverslip formed the base of an organ bath of 1 ml. volume (see Fig. 1). Solutions at 35° C, flowed through the path at approximately one bath volume per minute; the inflow and outflow were controlled by two roller pumps (Watson Marlow).

After mounting the chamber on the stage of an inverted compound microscope (Reichart, Biovert) both the gross organization and the outlines of individual

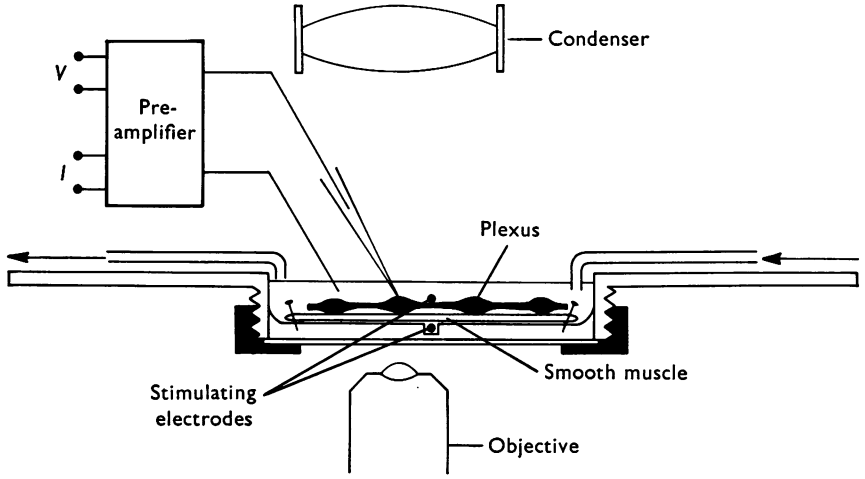

Fig. 1. Diagram of organ bath and recording arrangement. The preparation was pinned to a thin layer of silicone rubber and viewed from below using a compound microscope. Transmural stimuli were applied using fine platinum electrodes. See text for explanation.

neurones which lay in the nodes of the plexus could be observed. Using a conventional micromanipulator (Leitz) an electrode could be made to approach a cell before penetration. In all successful experiments a well-defined nerve cell could be seen. If penetrations were attempted in areas of poor definition or in the band of tissue on the outer edges of a node of the plexus no recordings from nerve cells could be made. In most cases when the electrode touched the cell or the sheath covering it, that cell was pushed to one side. This has been a major difficulty in making recordings from these neurones and this problem has not been overcome. Attempts to pin the preparation locally or to increase the tension at which the muscle layer was pinned were unsuccessful as this caused excessive spontaneous movements by the smooth muscle.

Micro-electrodes for intracellular recording were filled with 2M-KCl and had resistances of 80–150 MΩ; these were coupled to the recording apparatus (Tektronix 565) by a unity gain preamplifier (W.P.I.). The preamplifier allowed currents to be injected through the recording electrode either to stimulate an impaled cell directly or to estimate its electrical properties. As the resistance of the electrodes frequently appeared to alter on penetration the method suggested by Martin & Pilar (1963) was used to restore 'bridge' balance. No quantitative data were gathered if the electrode had a resistance greater than 100 MΩ.

In experiments where the preparation was stimulated transmurally, a fine platinum stimulating electrode was inserted into the base of the organ bath; a second electrode

was placed above this (see Fig. 1). The stimulus artifact was reduced by coating all but a fine strip of each of the electrodes with Araldite. In most experiments electrodes were placed at the oral end of the preparation.

The physiological salt solution had a composition (mM): NaCl, 120; KCl, 5; $CaCl_2$, 2·5; $MgCl_2$, 1; NaH_2PO_4, 1; $NaHCO_3$, 25; glucose 11. In the experiments involving manganese chloride either 3/4 $NaHCO_3$ were replaced by NaCl or the entire $NaHCO_3$ was replaced by Tris; pH was adjusted to 7.2.

In most experiments the smooth muscle was quiescent for some 2–3 hr after dissection. After this time, or earlier if the preparation had been either damaged or excessively stretched, the muscle layer became spontaneously active. This activity was prevented by adding isoprenaline bitartrate (1×10^{-7} to 1×10^{-6} g/ml.) to the physiological saline; in control experiments this had no effect on the properties of the neurones. When the preparation was to be stimulated transmurally atropine sulphate (1×10^{-7} g/ml.) (Burroughs Wellcome Ltd.) was added to the tissue fluid to prevent excitation of the smooth muscle layer (see below). Other drugs used were tetrodotoxin (Calbiochem Ltd.) and tubocurarine chloride (Burrows Wellcome Ltd.).

RESULTS

Although individual neurones were clearly visible and generally had diameters between 20 and 40 μm, it was difficult to obtain intracellular recordings from them. This was probably due to the elasticity of both the smooth muscle layer on which the plexus rested and the connective tissue covering its surface. Successful impalement of a neurone was indicated by a negative deflexion on the voltage recording trace which was accompanied by a brief burst of action potentials. Similar observations have been reported during the impalement of mammalian autonomic ganglion cells (Blackman, Crowcroft, Devine, Holman & Yonemura, 1969). The burst of action potentials was usually limited to four or five and rarely to one. After this, the membrane potential stabilized at a value of 40–60 mV (inside negative). It would be unwise to take these values as a meaningful measure of resting membrane potential because recordings could only be made with high resistance electrodes which had large and variable tip potentials. Moreover, the degree of electrical shunting around the electrode once it was inside the cell could not be determined.

In some cells the burst of action potentials caused by impalement did not cease but continued for several minutes without any noticeable change in the frequency or the amplitude of the action potentials. Others, which will be referred to later as 'afterhyperpolarizing' or AH cells, continued to fire either single or pairs of action potentials which were separated by intervals of 3–5 sec of silence. If a prolonged hyperpolarizing current (1×10^{-11} to 1×10^{-10} A) was passed through the recording electrode the discharge of action potentials was prevented, leaving a completely flat baseline. No residual changes in potential analogous to pacemaker potentials were ever recorded from any cell. In cells where the firing of action

potentials was continuous, hyperpolarization occasionally revealed low frequency spontaneous synaptic activity. This did not differ from the random spontaneous synaptic discharge occasionally recorded from an otherwise quiescent cell. There are reasons for thinking that these potentials are due to a release of packets of acetylcholine from presynaptic terminals (see below).

In two cells, spontaneous activity was recorded which appeared to have resulted from the invasion of the ganglion cell body by an action potential arising in one of its processes. When these cells were hyperpolarized the action potential no longer propagated into the soma but was blocked, leaving a residual electrotonic potential. These potentials had a rapid rising phase (less than 1 msec); successive potentials did not fluctuate in amplitude; and their amplitude was reduced in a graded manner by making the membrane potential more and more negative. Similar potentials have been observed in response to transmural stimulation of the plexus (see below).

It seems most likely that those cells in which the discharge of action potentials caused by impalement did not cease abrupty had been permanently damaged by the electrode so that their resting membrane potentials were reduced to threshold for firing of action potentials; we have found no evidence of pacemaker activity in any of the neurones in the myenteric plexus studied so far. It would appear that few, if any, of these nerve cells are normally spontaneously active under the conditions of our experiments. This is in contract with the observation of Ohkawa & Prosser (1972*a*) on the myenteric plexus of the cat. This may be a species difference or may result from the fact that in the experiments on the cat, unlike ours on the guinea-pig, the plexus remained attached to the circular muscle layer and was still in communication with the submucosal plexus.

Electrical properties of neurones

Small inward (hyperpolarizing) current pulses (90–200 msec) were passed through the recording electrode. Fig. 2 shows two examples of the relation between current and change in membrane potential after this had reached a steady state. Input resistance was determined from the slope of these curves for small increases in membrane potential; a range of values from 125 to 250 MΩ was obtained. Electrotonic potentials had a very slow time course and decayed to 1/e of the steady state value in about 20 msec (range 17–24 msec).

As shown in Fig. 2 input resistance fell when the cells were hyperpolarized by 20–40 mV. In skeletal muscle this phenomenon of anomalous rectification is due to an increase in potassium permeability as the potassium equilibrium potential is approached (Adrian, Chandler & Hodgkin, 1970). If the anomolous rectification observed in myenteric neurones has

a similar basis it seems unlikely that the values for resting membrane potential have been made grossly inaccurate by changes in micro-electrode tip potential.

As will become apparent, there are at least two distinct types of cells in the myenteric plexus, their differences being characterized by their regenerative responses to depolarizing current and their responses to transmural stimulation. However, there was no difference between the ranges of values obtained for input resistance and the time course of their electronic potentials. An example of the current–voltage relation for each of the two cell types is shown in Fig. 2.

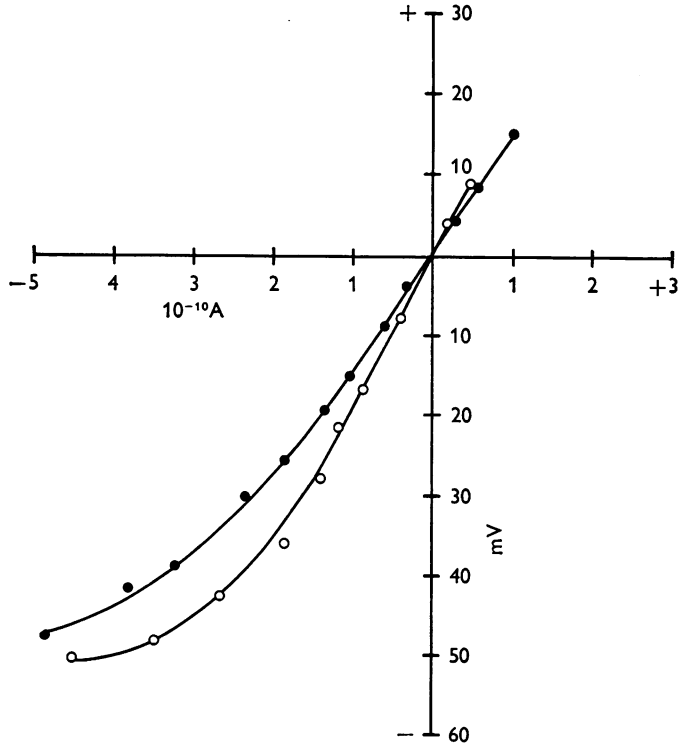

Fig. 2. The relation between steady changes in membrane potential and current intensity for the two different types of cells in the myenteric plexus. Current–voltage curves for an AH cell and a S cell are illustrated with filled and open circles respectively.

Action potentials and afterpotentials

Outward (depolarizing) current pulses of less than $0{\cdot}5 \times 10^{-10}$A elicited graded potentials. As the current intensity was increased an action potential was initiated. With pulse widths of 60 msec, threshold currents ranged from 1×10^{-10} to 3×10^{-10}A. In experiments where pulse width was

progressively shortened an action potential could be elicited with pulses as short as 2 msec. The threshold depolarization necessary to initiate an action potential ranged from 5 to 20 mV. Action potentials had amplitudes ranging from 60 to 95 mV; after the peak of depolarization there was a

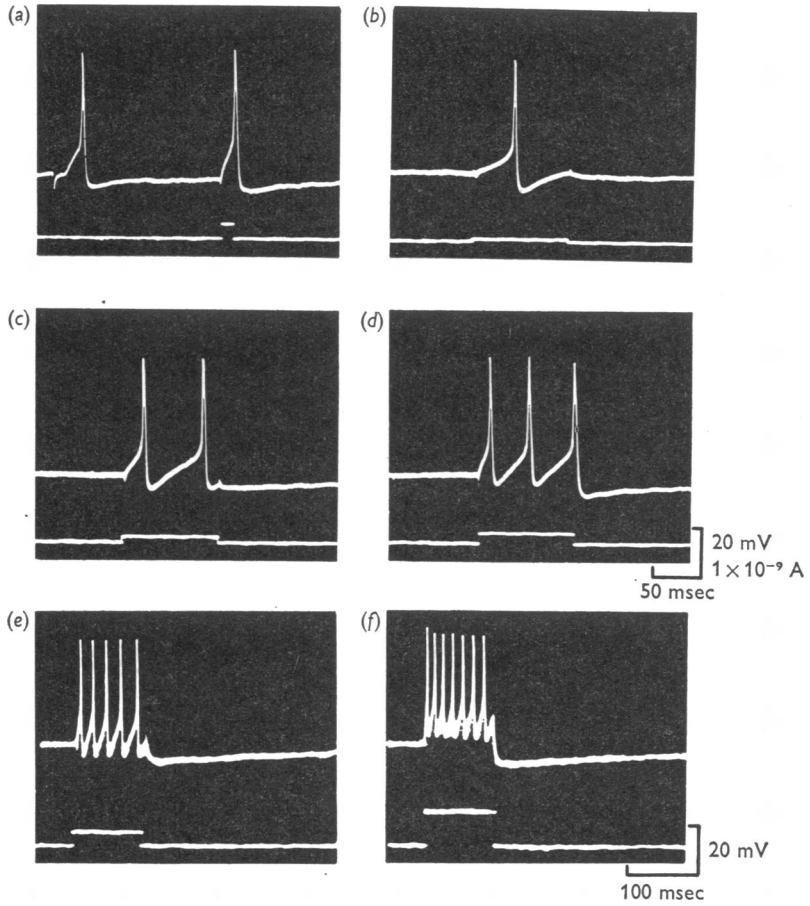

Fig. 3. Action potentials generated by a S cell. In Fig. 3*a* the first action potential was initiated by stimulating the preparation transmurally, the second was initiated by passing a brief current pulse through the recording electrode. The effect of increasing the intensity of current is shown in Fig. 3*b–f*. The upper trace records the intracellular potential; the lower trace records the duration and intensity of the current pulse.

rapid decay followed by a brief undershoot. In a few cells the decay of the action potential was complex, a slight plateau occurring on the falling phase.

In approximately two-thirds of the cells studied so far the membrane

potential returned to its resting value after the brief undershoot, and remained so until a second current pulse was passed through the recording electrode. If these cells were stimulated with longer or more intense current pulses then the cells could be made to fire repetitively at rates of up to 70/sec (see Fig. 3).

The second type of cell, as shown in Fig. 4, responded to brief outward currents, as described above, but the undershoot was followed by a persistant afterhyperpolarization; this increase in membrane potential was only observed if the current pulse was of sufficient intensity to initiate an

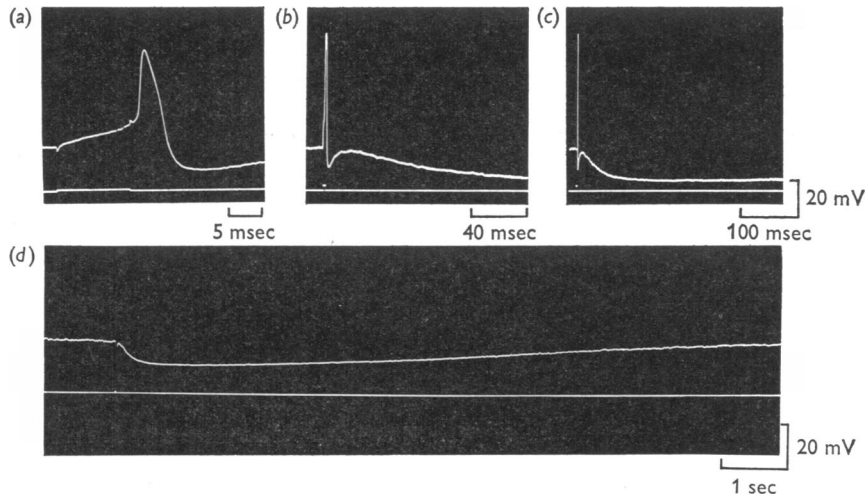

Fig. 4. Action potentials generated by an AH cell during the passage of current through the microelectrode. The responses of four cells are illustrated at different recording speeds. Only the peak of the action potential is seen in Fig. 4*d*.

action potential. The hyperpolarization had a maximum amplitude ranging from 6 to 20 mV which was reached some 200 msec after the action potential; it lasted for up to 20 sec in some cells but more commonly for about 10 sec (see Fig. 4). An increase in the duration and intensity of the current pulse (from 10 to 100 msec, 3×10^{-10} to 6×10^{-10} A) made it possible to drive two, three or occasionally four action potentials in these cells, as shown in Fig. 5. However, even if current pulses of more than 100 msec duration (Fig. 5*d*) were used, no further action potentials could be initiated. This was so even if the intensity of the current was further increased by an order of magnitude. Because of their characteristic afterhyperpolarization these cells will be referred to subsequently as AH cells.

The amplitude and duration of the afterhyperpolarization was increased

if it was preceded by two rather than a single action potential. As shown in Fig. 5 the delay before its onset was not altered nor was there any evidence of 'steps' during its onset when it was preceded by more than one action potential.

The afterhypolarization was associated with a marked decrease in the excitability of the cell. This was already indicated by their inability to give a sustained discharge of action potentials during prolonged depolarization.

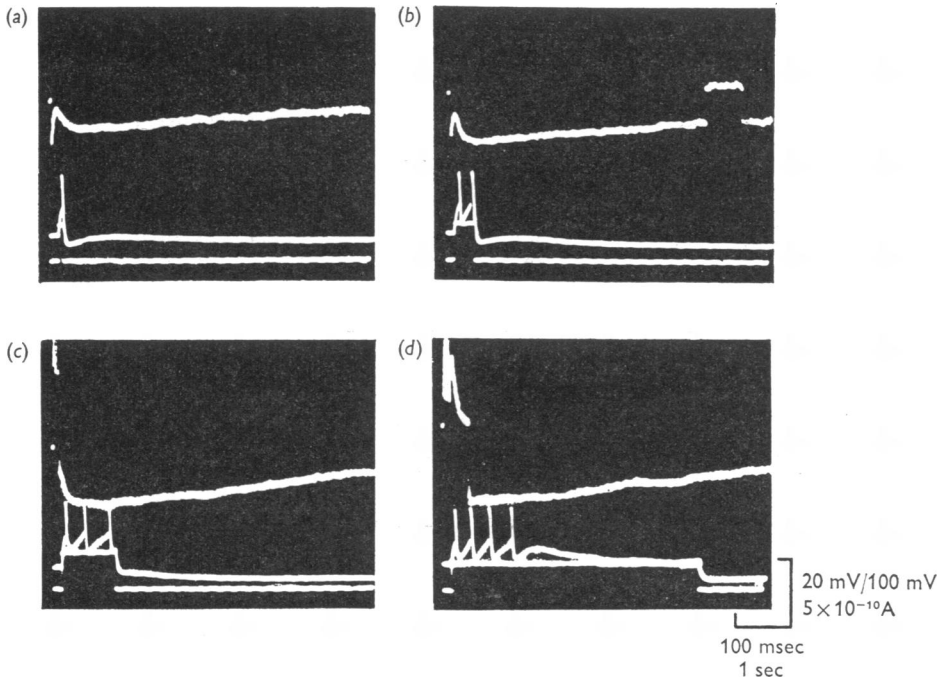

Fig. 5. Membrane potentials recorded from an AH cell during injection of current pulses of increasing duration. Each frame shows three simultaneous recordings. The lower traces record the membrane potential and the current intensity, each at the same speed. The upper trace records the membrane potential at a slower speed and higher amplification. A 10 mV calibration pulse is shown on Fig. 5*b*.

If a second outward current pulse was applied during the early stages of the afterhyperpolarization it was not possible to initiate a second action potential. Only as the afterhyperpolarization had started to decay could a further action potential be set up. The change in excitability appeared to result from a fall in the input resistance of the cell as well as the increase in membrane potential. The change in input resistance is shown in Fig. 6. In this experiment pulses of inward current were passed through the recording electrode at intervals of 1 sec. At the arrow, current polarity was

reversed and an action potential was initiated; the input resistance was subsequently reduced but returned to normal as the membrane potential returned to its resting value. The decrease in input resistance was associated with a fall in the noise level of the recording.

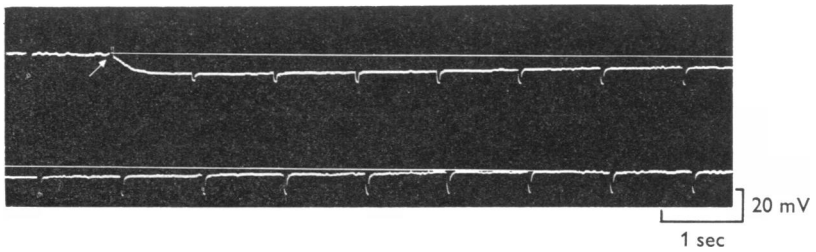

Fig. 6. The effect of initiating an action potential in an AH cell on the input resistance of that cell. Inward current pulses were passed through the micro-electrode at 1 Hz. The current pulse was reversed at the arrow and a single action potential is initiated. The upstroke of the action potential is not seen under these recording conditions (continuously moving film).

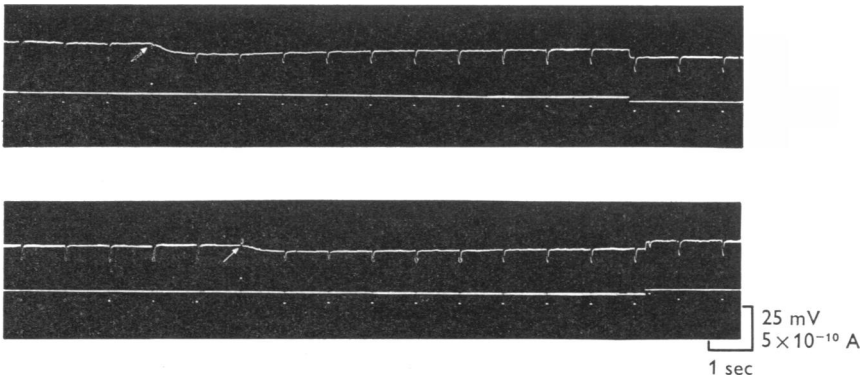

Fig. 7. The effect of membrane polarization on the input resistance of an AH cell and upon the amplitude of the afterhyperpolarization (photographed on moving film). Action potentials were initiated where indicated by the arrows. The membrane potential was changed by passing a prolonged inward current through the recording electrode.

Since these neurones show anomolous rectification it seemed possible that the decrease in input resistance could be due to the increase in membrane potential. This possibility was tested by changing the membrane potential with current pulses of long duration (20–30 sec). In Fig. 7 the membrane potential was displaced to a value slightly more negative than that attained during the peak of afterhyperpolarization following an action potential. Brief inward current pulses were passed repetitively (1 Hz) both before and during the long current pulse. The amplitude of the

voltage change produced by these brief current pulses was not altered. Evidently such a change in membrane potential was in itself unable to produce a change in input resistance. More intense currents, which carried the membrane potential to values 25–30 mV negative to the cell's resting membrane potential, did however cause the membrane resistance to fall. Thus we conclude that the afterhyperpolarization is associated with a fall in input resistance which cannot be accounted for by the passive electrical properties of these cells.

The amplitude of the afterhyperpolarization was influenced by the membrane potential. In a series of experiments similar to that described above, long current pulses (20–30 sec) were used to change the membrane potential; brief current pulses (60 msec, 1 Hz) were passed through the recording electrode to estimate changes in input resistance. Action potentials were initiated either by reversing the polarity of a brief pulse or by injecting an outward current pulse from a second stimulator. These experiments showed that the amplitude of the brief undershoot following the action potential and the afterhyperpolarization were reduced in a similar way. At hyperpolarizations of between 20 and 35 mV (five cells) both the undershoot and afterhyperpolarization were abolished. As noted previously at these levels of membrane potential the input resistance falls as a result of anomolous rectification. But it was still possible to demonstrate that a further decrease in input resistance followed each action potential (see Fig. 7).

The afterhyperpolarization did not alter in amplitude even when cells had been impaled for up to 90 min; that it, is was unaffected by the leakage of chloride ions from the micro-electrode into the cell. Attempts to investigate the effect of changes in potassium concentration were unsuccessful as either a reduction or an increase in external potassium concentration caused the smooth muscle to which the plexus was attached to become spontaneously active. In view of the evidence that the undershoot in frog ganglion cells (Blackman, Ginsborg & Ray, 1963*a*) and mammalian ganglion cells (M. E. Holman, unpublished observation) is due to an increase in potassium conductance (G_K) it seems possible that the afterhyperpolarization may have a similar basis.

Responses to transmural stimulation

Contractile responses of the longitudinal smooth muscle occurred in response to single stimuli of 0·1–2·0 msec duration. Since these were abolished by atropine (10^{-7} g/ml.) it was concluded that such stimuli caused stimulation of myenteric neurones leading to the release of acetylcholine (ACh) onto the smooth muscle. More intense stimuli, whose duration was in excess of 10 msec, caused the smooth muscle to contract in the presence of atropine. In the experiments described below, stimulus duration was limited to less than 2 msec and atropine (10^{-7} g/ml.) was present to prevent contractions which dislodged the microelectrodes.

Again the two types of cells lying in the plexus could be distinguished. All but two of more than sixty AH cells studied so far showed no response whatsoever to transmural stimulation. The two cells which gave a response were less than 1 mm from the stimulating electrode. Transmural stimulation initiated an action potential in an all-or-nothing manner which could be prevented from invading the soma by hyperpolarizing the cell by some 10–15 mV. A residual electrotonic potential of rapid rise time (less than 1 msec) was then observed. As the membrane potential was made more negative by increasing the intensity of the current flowing through the microelectrode the amplitude of the residual potential decreased in a graded manner. These observations could be explained if a process of the AH cell had been stimulated by the transmural electrodes and the action potential from this process was invading the soma 'antidromically'. Action potentials initiated by transmural stimulation were followed by the characteristic afterhyperpolarization.

Attempts were made to excite these two cells 'antidromically' during the afterhyperpolarization following an action potential. Although the cell soma could not be excited directly electrotonic potentials were observed in response to transmural stimulation. Their time course was similar to that of the potentials observed when an antidromic impulse was prevented from invading the soma by a prolonged current inward pulse. This finding indicates that the processes of AH cells do not undergo a decrease in excitability following an action potential.

Synaptic activity could always be recorded from the other type of cell when preparations were stimulated transmurally. In order to distinguish them from AH cells, those with synaptic input will be referred to as S cells. At low stimulus strengths a single synaptic potential was initiated (Fig. 8*a*) whose amplitude fluctuated during repetitive stimulation. As the stimulus strength was increased, additional synaptic potentials were evoked which summed with each other until depolarization reached threshold for the initiation of an action potential. Still further increases in stimulus intensity shortened the delay before threshold was reached; the amplitude of the action potential was reduced and the undershoot was abolished or replaced by a more persistent depolarization. Occasionally two action potentials were initiated by a single stimulus. The most likely explanation for this sequence of events is that an increasing number of presynaptic fibres were being recruited leading to a greater short circuiting action by the transmitter (Blackman, Ginsborg & Ray, 1963*b*). The persistent afterdepolarization may result from a slow inactivation of transmitter or from a temporal dispersion in the release of transmitter from many synaptic terminals.

The initial burst of synaptic activity, such as that shown in Fig. 8, was

often followed some 20–50 msec later by a delayed response consisting of one or more synaptic potentials. The time course and other characteristics of the late synaptic potentials were identical with those of the early ones. Fig. 9*a* is an example of a cell in which late synaptic activity exceeded threshold for the initiation of an action potential. Late synaptic potentials occurred at a constant latency and thus it is unlikely that they were due

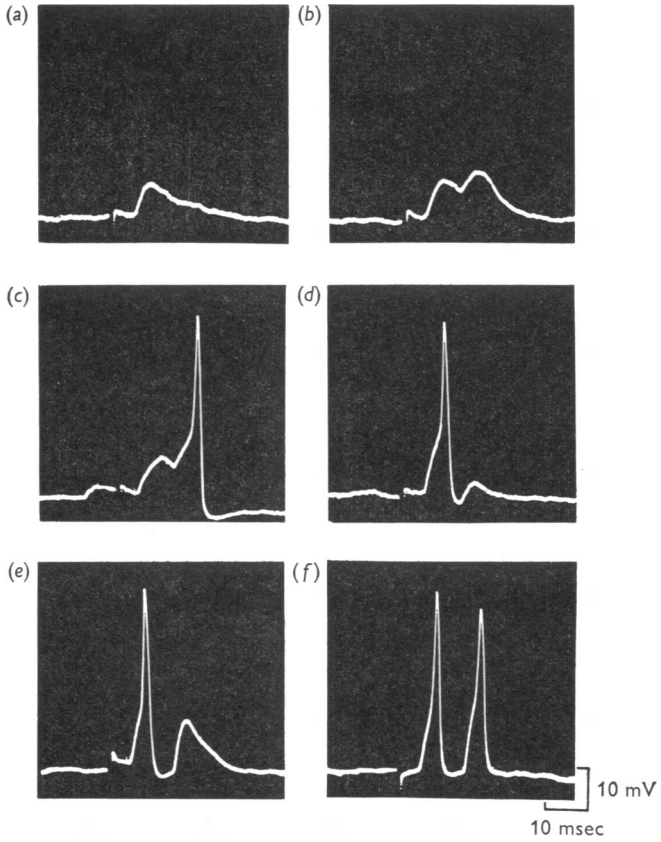

Fig. 8. Synaptic responses recorded from an S cell during progressive increases in stimulus strength (transmural stimulation, see text). The stimulus pulse width was 1 msec throughout; the stimulator output voltage was increased from 5 to 22 V during the recording period.

to the spontaneous release of transmitter from terminals whose excitation had caused the early response. The late potentials are more likely to be due to release of ACh from presynaptic fibres having a very slow conduction velocity or from terminals which had been excited by a polysynaptic pathway.

In a few experiments, transmural stimulation caused an action potential in an S cell in the absence of any synaptic response. As described above for AH cells, hyperpolarization revealed a residual electronic potential with a rapid rising phase. This response was not blocked by turbocurarine (up to 1·44 μM) and it was assumed that a cell process had been activated

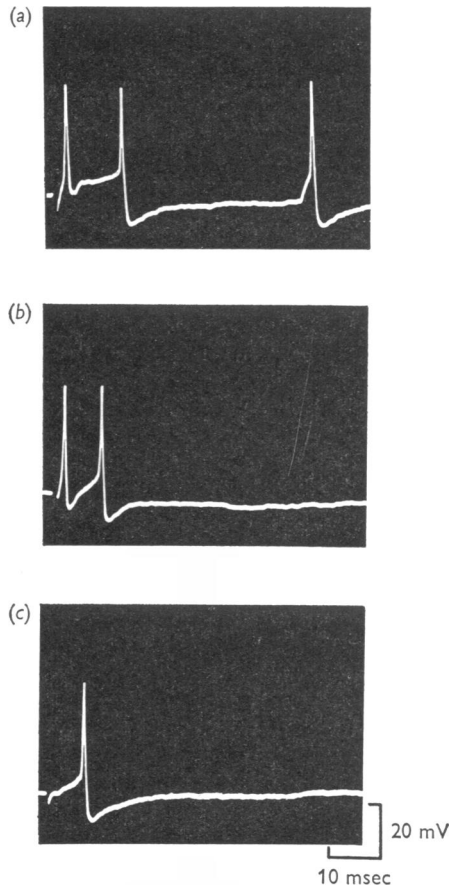

Fig. 9. The effect of tubocurarine on the early and late synaptic potentials recorded from an S cell. The late potential was abolished in an all-or-nothing manner (Fig. 9*b*) 3 min after changing to a solution of tubocurarine (0·144 μM). Only a single action potential was evoked 5 min after changing to tubocurarine solution (Fig. 9*c*). Stimulus strength remained constant throughout.

antidromically and not via a distant axo-dendritic or axo-axonal synapse. Responses were often recorded from S cells which appeared to result from a mixed synaptic and antidromic input.

Throughout these experiments the transmural stimulating electrodes

were positioned approximately at right angles to the longitudinal axis of the intestine. There were no obvious differences in the responses obtained from S cells which were either oral or aboral with respect to the stimulating electrodes. Orthodromic, antidromic or mixed responses were observed regardless of the position of cell. However it must be pointed out that no attempt has been made so far to analyse the frequency of occurrence of these responses in relation to the position of the cell. All S cells studied, which were up to 1·2 cm from the stimulating electrodes, could be excited synaptically; many could also be excited antidromically. In contrast, only 2 AH cells responded to transmural stimulation and these were probably excited antidromically. Both these AH cells were within 1 mm of the electrodes.

Spontaneous synaptic potentials were also recorded from S cells. They had a similar time course to single evoked synaptic potentials (Fig. 8*c*), and they were abolished by tubocurarine (0·144 μM). In most cells their frequency was low, of the order 1–5 per minute. This did not allow sufficient data to be collected for analysis of the distribution of their amplitudes, or their frequency. No spontaneous synaptic potentials have even been recorded from AH cells.

Effect of tubocurarine on synaptic activity

All post-synaptic activity in response to transmural stimulation was abolished by tubocurarine (0·144–1·44 μM). In contrast with its action on autonomic ganglia (Blackman *et al.* 1969) tubocurarine completely blocked synaptic potentials in the myenteric plexus, within 2–5 min. This rapid action may result from the relatively unhindered diffusion of the drug to the neurones of the plexus since they form a single layer of cells (Gabella, 1972). Alternatively, the nicotinic receptors of myenteric neurones may differ from those of autonomic ganglia.

During the onset of action of tubocurarine it was apparent that the drug had different effects on the early and late synaptic activity described above. The intensity of early synaptic activity was reduced in a gradual way, as shown in Fig. 9. However, late synaptic activity, although depressed by the drug, suddenly disappeared in an 'all-or-nothing' manner. The simplest explanation for this finding is that the late synaptic activity is due to the release of ACh from interneurones which are excited by cholinergic terminals; that is, the late synaptic activity is due to a polyneural cholinergic pathway.

Action potentials and the late afterhyperpolarization recorded from AH cells in response to direct stimulation were unaffected by perfusing with tubocurarine (1·44 μM) for at least 15 min.

Effect of tetrodotoxin

Since we were interested to know if the afterhyperpolarization in AH cells was dependent upon the initiation of an action potential or merely upon intense depolarization of the cell membrane we decided to study the effect of tetrodotoxin on myenteric neurones. It became apparent that AH and S cells could be further differentiated by the action of this drug. Tetrodotoxin (1×10^{-6} g/ml.) abolished the action potentials of S cells within 3 min, as shown in Fig. 10. Synaptic potentials were also blocked

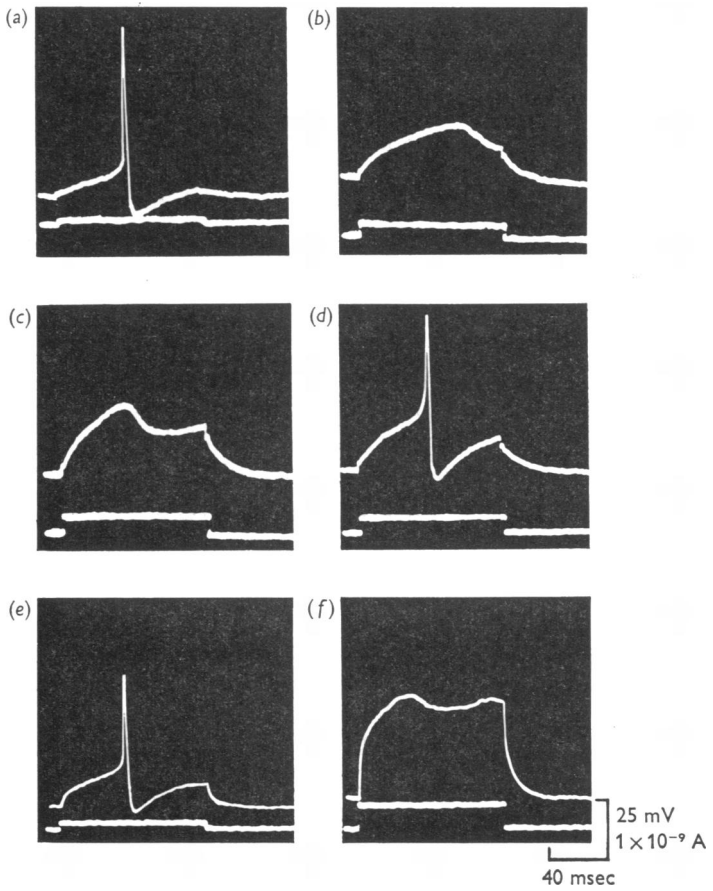

Fig. 10. Effect of tetrodotoxin on action potentials evoked in AH and S cells. Fig. 10(*a–d*) responses recorded from AH cell, before (*a*) and during the period 10–11 min (*b–d*) after changing to solution containing tetrodotoxin (1×10^{-6} g/ml.). The effect of tetrodotoxin on an S cell is shown in Fig. 10(*e–f*). The recording (*e*) was taken immediately before changing to tetrodotoxin; Fig. 10(*f*) was recorded 3 min later.

though these persisted for a short time after it was no longer possible to initiate an action potential by direct stimulation. Similar observations have been reported for the frog skeletal neuromuscular junction (Katz & Miledi, 1967).

Action potentials could still be evoked in AH cells after exposure to tetrodotoxin (1×10^{-6} g/ml.) for at least 1 hr. The results of such an experiment are shown in Fig. 10. After 10 min exposure the threshold of the action potential was increased by 15 mV, and its amplitude and rate of rise were slightly reduced. No further changes were observed in the action potential during the following 20 min exposure to tetrodotoxin. Some AH cells gave abortive responses in the presence of tetrodotoxin, in contrast with their normal 'all-or-nothing' behaviour. Outward current pulses of increasing intensity caused regenerative responses of increasing amplitude until a maximal regenerative response was initiated (Fig. 10*b* and *d*). The afterhyperpolarization was unaffected by tetrodotoxin.

Effect of changes in ion concentration on AH cells

In a series of preliminary experiments the ionic requirements for the generation of tetrodotoxin-resistant action potentials were examined. The amplitude of the action potential was unaffected by a fourfold reduction in the extracellular sodium concentration (106 mM-NaCl were replaced with sucrose). The result of such an experiment is shown in Fig. 11*a* and *b*. When a more drastic reduction in external sodium concentration was attempted the smooth muscle became spontaneously active (Holman, 1957). The tetrodotoxin-resistant action potentials were sensitive to changes in extracellular calcium concentration. An increase in concentration from 2·5 to 10 mM caused an increase in the amplitude of the action potential (Fig. 11*e* and *f*). Decreasing the calcium concentration to 0·5 mM decreased the action potential amplitude (Fig. 11*c* and *d*). Although more experiments are required to give quantitative data the present results suggest that calcium ions may be involved in the initiation of action potentials in the presence of tetrodotoxin.

The action potentials of barnacle muscles, which are due to an increase in permeability for calcium ions, are blocked by manganese ions. The effect of manganese (5 mM) in the presence of tetrodotoxin is shown in Fig. 12. In this and four other experiments the action potential was abolished. This effect appeared to be irreversible as no regenerative activity returned after washing with normal solution for 20 min.

During the onset of the action of manganese, in the presence of tetrodotoxin, it was possible to initiate action potentials which were not followed by an afterhyperpolarization. Lower concentrations of manganese (0·1–0·2 mM) which caused only a small reduction in the amplitude of the action

potential abolished the afterpotential. In contrast with the action of higher concentrations, this effect of manganese was readily reversible. Since the afterhyperpolarization was blocked so readily by manganese ions it was decided to study their action in normal solution.

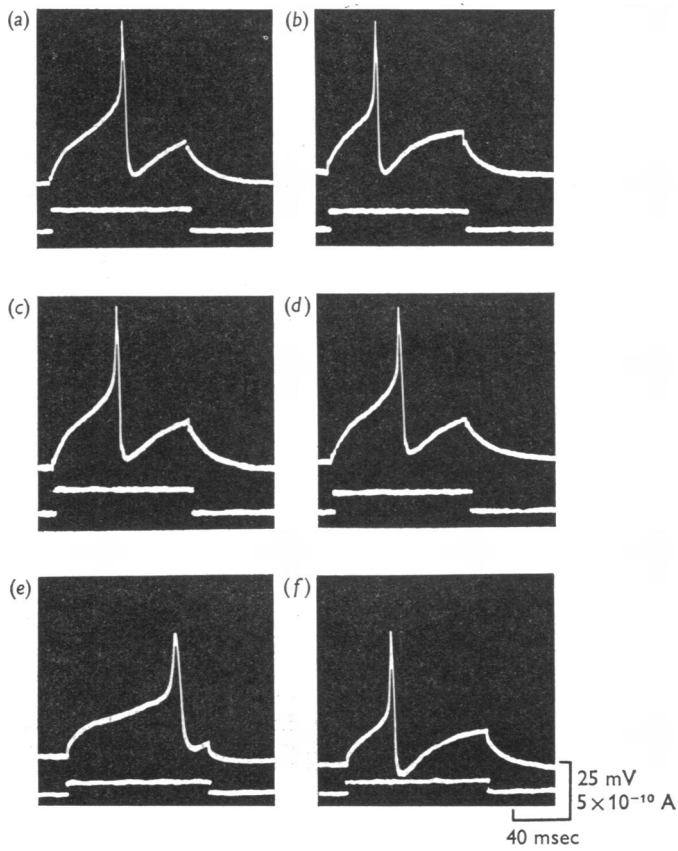

Fig. 11. Effect of changes in extracellular sodium and calcium concentrations on action potentials generated by AH cells in the presence of tetrodotoxin (1×10^{-6} g/ml.). Action potentials initiated before (Fig. 11*a*) and after (Fig. 11*b*) reducing the extracellular sodium concentration from 146 to 40 mM. The effects of increasing the extracellular calcium concentration to 10 mM (Fig. 11*d*) and decreasing it to 0·5 mM) (Fig. 11*f*) are shown: control records are shown on the left.

Manganese ions (0·2 mM) caused a reduction in resting membrane potential of 5–10 mV and an increase in input resistance. Action potentials of normal amplitude were observed but the afterhyperpolarization was abolished. The results of such an experiment are illustrated in Fig. 13. In the presence of manganese ions there was no sign of the increase in

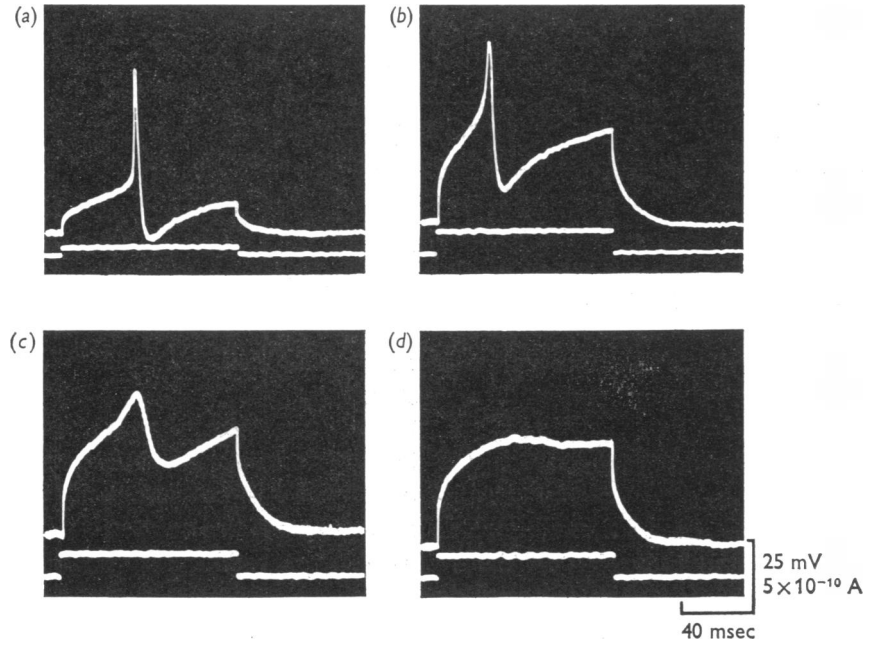

Fig. 12. Effect of manganese ions on action potentials initiated in AH cells tetrodotoxin (1×10^{-6} g/ml. present). Fig. 12*a* shows an action potential recorded before changing to a solution containing manganese ions (5 mM). Fig. 12*b–d* are recordings made 1 min, 3 min and 5 min after changing to this solution.

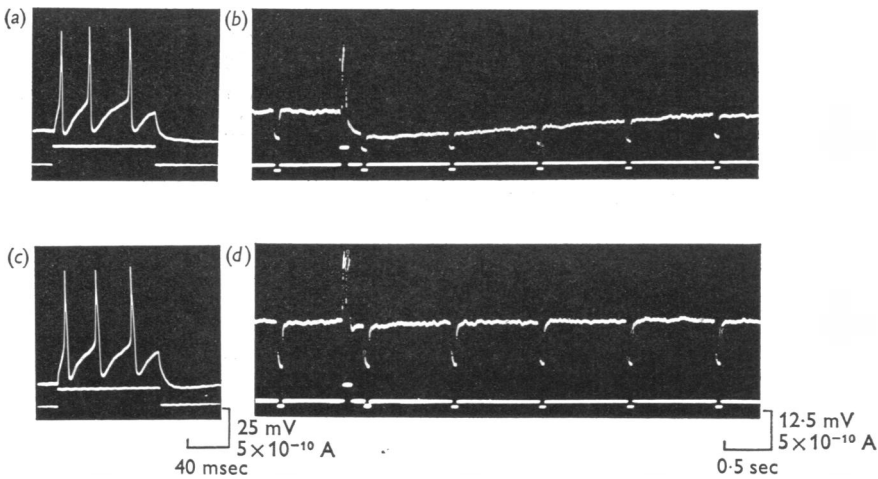

Fig. 13. Effect of manganese ions of action potentials and the afterhyperpolarization recorded from an AH cell. Figs. 13*a* and *b* show three action potentials and the associated after hyperpolarization immediately before changing to a solution containing manganese ions (0·2 mM). In Figs. 13*c*, *d*, the evoked responses of the same cell in the presence of manganese are shown. In addition to preventing the afterhyperpolarization the cell resting membrane potential was decreased and the input resistance increased.

membrane conductance which normally followed an action potential (Fig. 13*b*, *d*). If it is accepted that manganese ions depress membrane permeability for calcium ions then it appears that the increase in conductance is caused by an increase in intracellular calcium concentration.

DISCUSSION

At least two types of neurones are located in the nodes of the myenteric plexus of guinea-pig duodenum. One group of cells, which we have referred to as S cells, have an extensive synaptic input and their electrical properties are similar to those of mammalian autonomic ganglia (Blackman & Purves, 1969; Crowcroft & Szurszewski, 1971). The second group have been called AH cells in accordance with the large afterhyperpolarization which followed each action potential. We have been unable to demonstrate any synaptic input onto these cells. The passive properties of the two groups of cells appear to be similar. The values obtained for input resistance and the time course of the electrotonic potential are somewhat higher than those usually reported for autonomic ganglia (Blackman & Purves, 1969).

The synaptic potentials recorded from S cells were similar to those described for other autonomic ganglia. They were blocked rapidly and reversibly by tubocurarine and it would seem likely that they are due to the release of ACh. There is evidence that ACh is released from presynaptic terminals in the myenteric plexus of the guinea-pig (Paton & Zar, 1968). Although transmural stimulation would be expected to excite all the neural elements with the plexus we have been unable to find evidence for the release of any transmitter other than ACh which might act postsynaptically.

Many presynaptic fibres appeared to converge on to any one S cell. The latencies of synaptic potentials were dispersed over a wide range. This may be due to a variation of conduction velocities of presynaptic fibres and to the variability of the length of the pathway between the stimulating electrodes and the cell being studied. Langley (1922) suggested that the plexus may contain interneurones. The very long latency of some of the synaptic potentials could have resulted from synaptic delays during transmission at one or more interneurone.

The position of a neurone in relation to the stimulating electrodes did not noticeably influence the pattern of its response. Although the latency of synaptic potentials increased with distance there was no obvious decrease in synaptic activity even when the stimulating and recording electrodes were separated by 1·2 cm. This result is surprising in view of Bülbring & Tomita's (1967) observations on the inhibitory junction potentials recorded

from the taenia coli in response to excitation of non-adrenergic inhibitory neurones in the myenteric plexus (Bülbring & Tomita, 1967), to transmural stimulation. They found that inhibitory junction potentials could only be recorded from smooth muscle cells in close proximity of the stimulating electrodes. Either Bülbring & Tomita's method of stimulation did not excite the presynaptic fibres to these inhibitory neurones or we have failed so far to record from them. On the other hand, it seems likely that some of the S cells were cholinergic motoneurones to the smooth muscle as it is known that the evoked release of ACh can be reduced by ganglion blockade (Gillespie, 1968).

In view of the many reports that the myenteric plexus receives a dense adrenergic innervation (Jacobowitz, 1965) and that noradrenaline may excite neurones in cat intestine (Ohkawa & Prosser, 1972*b*) it is surprising that we could find no evidence for post-synaptic activity mediated by the release of noradrenaline. However Paton & Vizi (1969) have suggested catecholamines have a modulatory role and exert their action presynaptically by inhibiting the release of ACh. Support for such a role was obtained by Holman, Hirst & Spence (1972) who found that noradrenaline (NA) caused a reduction in the amplitude of evoked potentials but had no consistent effect on the electrical properties of the cell body. Similar findings have been reported by Nishi & North (1973). If NA acts presynaptically the effects of stimulating sympathetic nerves would only be apparent if the neurones of the plexus were undergoing continuous synaptic activity (see for example, Crowcroft, Holman & Szurszewski, 1971). It is evident that in the guinea-pig such synaptic activity is not the result of spontaneous firing of the neurones of the myenteric plexus but must result from stimulation of sensory receptors or from spontaneous firing of the neurones of the submucous plexus. The sympathetic inflow to the myenteric plexus must be stimulated selectively before its action can be clarified further.

As we have indicated, a second group of neurones (AH cells) was distinguished from S cells by their lack of synaptic input and the prolonged afterhyperpolarization following an action potential. The slow afterhyperpolarization was associated with a decrease in input resistance and evidence has been presented which suggests that it results from a prolonged increase in potassium permeability. The possibility that this prolonged increased in conductance was mediated by an inhibitory chemical transmitter was considered. Such a transmitter could have been released by a recurrent inhibitory pathway involving neighbouring AH cells, or by an AH cell liberating transmitter on to its own soma. Recurrent inhibition generally involves excitation of groups of cells rather than single ones but neither of these possibilities can be ruled out at present. However, magnesium ions did not block the conductance change and it persisted in the

presence of atropine, tubocurarine, phentolamine and isoprenaline (unpublished observations).

Since the afterhyperpolarization could be blocked by manganese, it seems possible that it was caused by an influx of calcium. Moreover, it was still possible to evoke action potentials in AH cells in the presence of tetrodotoxin; the tetrodotoxin-resistant action potentials were unaffected by gross changes in external sodium concentration. They were sensitive to changes in extracellular calcium concentration and were blocked by manganese. As a working hypothesis we suggest that in the absence of drugs depolarization of AH cells first causes an increase in G_{Na^+}. Such a conductance change can give rise to an action potential which is blocked by tetrodotoxin. In addition, depolarization causes a voltage dependent increase in $G_{Ca^{2+}}$ which can also lead to an action potential if the increase in G_{Na^+} has been prevented. The influx of calcium during the upstroke of the action potential in some way causes the afterhyperpolarization. As already pointed out, we cannot rule out the possibility that such an influx of calcium may cause the release of an inhibitory transmitter which acts on the membrane of the cell from which it was released. Alternatively, the influx of calcium may cause a prolonged increase in G_{K^+} by a direct action on the cell membrane: a similar mechanism has been proposed by Godfraind, Kawamura, Krnjević & Pumain (1971) to occur in cortical neurones.

It has not been possible so far to evoke any synaptic activity in AH cells by transmural stimulation of the plexus, and no spontaneous synaptic activity has ever been recorded from these cells. These findings suggest that AH neurones, in common with primary afferent neurones, may not be innervated. It is generally accepted that sensory neurones are present in the plexuses of the gastrointestinal tract, since the peristaltic reflex persists after the extrinsic nerve supply to a segment of intestine has been cut and allowed to degenerate. If AH neurones are sensory neurones then their characteristic afterhyperpolarization could be significant in relation to the signalling of sensory information. However it must be pointed out that in the two AH cells which could be excited antidromically, this behaviour did not appear to be characteristic of these processes. Alternatively, these cells may be excitable by short axons arising from neurones within the same node of the plexus, which were not excited by transmural stimulation. It this were so AH cells could be those which release non-adrenergic inhibitory transmitter on to smooth muscle; that is, these neurones may correspond with those that have been termed 'purinergic' by Burnstock and his colleagues (see Burnstock, 1972).

The role of both types of cell in the peristaltic reflex and other responses of the intestine cannot be defined until communication between neurones

within the plexus and their interaction with its smooth muscles have been investigated.

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